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Protection Branch Report of Test No. 4-64

USE OF SPORICIDES AND HEAT TO STERILIZE RESINS

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Use of Sporicides and Heat to Sterilize Resins

A major problem in the over-all project of developing sterilization procedures applicable to interplanetary spacecraft is that of sterilizing resins used to pot electronic assemblies. Since the resins themselves do not seem to possess self-sterilizing properties, an additional treatment appears necessary to insure that no microorganisms remain viable within the body of a resin or on the surface of solid objects coated by the resin. Two approaches to the solution of the problem have been investigated and are described in this report. One approach was to impregnate resins with a known sporicide and the other was to cure the resins at an elevated temperature. The resins used in the first approach were supplied by Jet Propulsion Laboratories and those for the latter by Goddard Space Flight Center.

MATERIALS AND METHODS

Resins

The five resins impregnated with a sporicide were: Epon 828 with Epocast 985, Epon 828 with Versamid 125, Epon 828 with Versamid 140, Epon 815 with Catalyst TETA, and Eccofoam FP with Catalyst 12-6. The five silicone resins subjected to heat curing were: RTV-20 with Thermolite-12, RTV-40 with Thermolite-12, RTV-60 with Thermolite-12, RTV-90 with Thermolite-12, and LTV-602 with Catalyst SRC-05. The curing temperature and time employed were 145° C and 24 hours as requested by Goddard Space Flight Center.

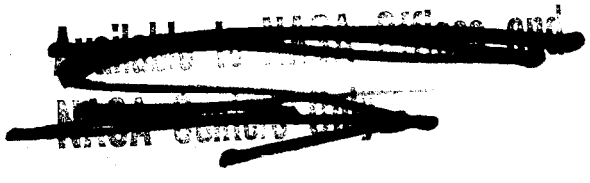
Sporicides

The sporicides that were added to the above resins were: 10 per cent ethylene imine in 14 per cent V/V aqueous methanol, 10 per cent ethylene imine in absolute methanol, undiluted ethylene imine, undiluted formalin, and undiluted beta-propiolactone.

Test Procedure

A detailed description of the test procedure used was given in a previous report 1/. In short, however, the sporicide impregnated resin samples were prepared by covering glass slides, previously contaminated with

1/ Protection Branch Report of Test No. 11-63: "Procedure for Evaluation of Self-Sterilizing Resins". 19 December 1962.



dry spores of Bacillus subtilis var niger, with the freshly mixed catalyst-monomer sporicide solution and allowed to cure at room temperature. After each curing period, i.e. about one day, one week and six weeks at room temperature, seven samples were exposed to ethylene oxide gas in a plastic chamber for six hours and then the samples were crushed as well as possible and placed in tryptose broth blanks, Three of the resin samples were assayed by the pour plate method to obtain a viable spore count. In addition, three contaminated glass slides containing no resin (controls) were assayed for viable spores in the same manner. The other four resin samples were used to test for sterility; two of these samples were cultured with the crushed glass and resin present in the broth media and the other two broth samples were subcultured to minimize any inhibitory effects that might be produced by the resin or sporicide.

The silicone resin samples were prepared by embedding glass slides contaminated with dry spores, in 1/2 inch depth of the monomer-catalyst mixture. One set of eight samples was cured for 24 hours at room temperature (about 25° C) and another set of eight was cured for 24 hours at 145° C. Four samples of each set of eight were used to obtain a viable spore count by the pour plate method and the other four were used to test for sterility in broth media as described above. However, two of the four samples for each method of testing were exposed directly to ethylene oxide gas; the other two were contained in a sealed jar within the chamber to prevent direct contact with the ethylene oxide. This procedure not only provided a means to determine whether ethylene oxide gas would penetrate and sterilize the interior as well as the exterior of the porous silicone resins but also provided a sterile atmosphere in the chamber in which to pulverize the samples. This procedure was also followed when the porous Eccofoam FP with Catalyst 12-6 was tested.

RESULTS AND DISCUSSION

The results obtained by the pour plate method on the resins are summarized in Tables I and II. Since there was no appreciable difference in the per cent recovery after a one day, one week or six week curing period, an average value is given (Table I). Some viable B. subtilis var niger were recovered from each resin-sporicide sample when assayed by the pour plate method. For most resin-sporicide combinations, both the cultured resin and glass samples and the subcultures also, showed bacterial growth after a seven day incubation period at 37° C. However, with Epon 815 with Catalyst TETA and ten per cent ethylene imine in absolute metanol or undiluted ethylene imine, there was no evidence of bacterial growth in either the cultured resin and glass samples or in the subcultures. All samples did support bacterial growth however when about 100 spores of B. subtilis var niger were later deliberately introduced to test for any bacteriostatic effect of the resin.

Table II shows that no bacterial count was obtained for resins cured at 145° C; moreover, the cultured resin-glass samples and the subcultures were also sterile, The results of the 25° C curing temperature indicated that some spore reduction but not serility, was achieved by the penetration of ethylene oxide gas into the porous silicone resins. Similar results were observed with samples of Eccofoam FP with Catalyst 12-6 that were exposed to ethylene oxide gas. Possibly, if a long exposure period was used, ethylene oxide gas would sterilize electronic assemblies thinly coated with a very porous resin.

Ethylene imine was the only promising sporicide for Epon-type resins. Gross changes in the appearance of the Epon resins occurred when formalin or beta-propiolactone was used. From these results, it appears that no microorganisms would remain viable in the resin or on the surface of the electronic assembly if potted with any of the five silicone resins and then cured at 145° C for 24 hours. With slightly more reservation the same statement also applies to Epon 815 with Catalyst TETA and ethylene imine either with or without methanol cured at 25° C.

Table I.

Per Cent Recovery of *B. subtilis* var *niger* Spores in Various Resins Impregnated With Sporicidal Chemicals

Resin	No. Sporicide	Per Cent Recovery: 10% ETI in 14% MeOH	10% ETI in Absolute MeOH	Undiluted ETI	Undiluted Formalin	Undiluted BPL
Epon 828 with Epocast 985	—	0.0003*	0.006*	0.96*	0.22**	4.7**
Epon 828 with Versamid 125	4.6*	—	0.12*	1.4**	0.68**	1.7**
Epon 828 with Versamid 140	2.9*	—	0.024*	0.22**	1.7**	3.2**
Epon 815 with Catalyst TETA	10.0**	—	0.0007*	0.00009*	0.42**	2.5**
Eccofoam FP with Catalyst 12-6	13.0*	—	2.4**	4.8**	—	—

* Average of 18 determinations.

** Average of nine determinations.

Note: (1) For the first three resins, the proportions of resin/catalyst and resin/catalyst/sporicide was 50/50 and 49/49/2 by weight respectively. For the last two resins, the proportion of resin/catalyst and resin/catalyst/sporicide was 10/1 and 89/9/2 by weight respectively.

(2) Per cent recovery based upon a control count of a few million spores/glass slide.

(3) Eccofoam FP with Catalyst 12-6 was not exposed to ethylene oxide gas.

Table II.

Per Cent Recovery* of *B. subtilis* var *niger* Spores in Silicone Resins After Curing for 24 Hours at 25° and 145° C

Resin	Per Cent Recovery			
	25° C		145° C	
	Exposed to ETO	Not Exposed	Exposed to ETO	Not Exposed
RTV-20 with Thermolite-12	0.0007	0.16	0	0
RTV-40 with Thermolite-12	0.000006	0.27	0	0
RTV-60 with Thermolite-12	0.000008	0.0076	0	0
RTV-90 with Thermolite-12	0	0.000006	0	0
LTV-602 with Catalyst SRC-05	0.00005	4.6	0	0

* Based upon a control count of a few million spores/glass slide not coated with the resin.

Note:
(1) Each entry is an average of two determinations.
(2) Proportion of RTV resin/Thermolite-12 was 200grams/25 drops.
(3) Proportion of LTV resin/SRC-05 was 200 grams/30 drops.